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Abstract:

Purpose: To determine if the addition of Go6976 to vaccine protocols will inhibit neu specific tolerance and enhance immunotherapy for breast cancer.
Scope: In the Her-2/*neu* model of spontaneous breast cancer the immune system of these transgenic mice are tolerant to the neu protein. While immunity to neu can be demonstrated in the neu-transgenic mice (partial breaking of tolerance), this immunity is inadequate in terms of preventing the spontaneous development of tumors and preventing death from tumor challenge. Our experiments involve concomitantly treating mice with the PKC inhibitor Go6976 during tumor vaccine therapy in order to prevent tolerance induction and enhance immunotherapy.
Findings: By combining our regimen with a dose of cytoxan we can promote survival of tumor bearing mice when compared with no treatment, vaccine alone or vaccine + cytoxan. We believe that the cytoxan eliminates T regulatory cells. In particular, this combination is very effective in inhibiting tumor growth in the early period post-tumor challenge. Based on these findings we have concludes that extended administration of Go6976 can enhance the efficacy of this regimen even further.
Significance: These data support the notion that the novel combination of PKC inhibitor + vaccine can enhance the efficacy of tumor vaccines.

Introduction:

In the Her-2/*neu* model of spontaneous breast cancer development it is clear that the immune system of these transgenic mice are tolerant to the neu protein (1-3). In this model not only does the overexpression of neu lead to tumorogenesis but the neu protein is the target of both humoral and cellular immunity which prevent tumor-induced death in the non-transgenic mice (1, 4). Indeed, while immunity to neu can be demonstrated in the neu-transgenic mice (partial breaking of tolerance), this immunity is inadequate in terms of preventing the spontaneous development of tumors and preventing death from tumor challenge. We have demonstrated *in vitro* that the PKC inhibitor Go6976 has the ability to selectively inhibit TCR induced tolerance induction while only minimally inhibiting T cell activation. We hypothesize that the addition of Go6976 to vaccine protocols will inhibit the reinduction of neu specific tolerance and thus facilitate immune mediated protection against the development of spontaneous breast cancer development and tumor challenge.

Body:

Optimization of drug delivery:

There was essentially no data concerning *in vivo* use of Go6976. Thus, a major challenge of this proposal (Task 1) was to determine the optimal dose for inhibiting tolerance *neu*-specific tolerance induction in this model. Beginning with an initial educated guess of 5mg/kg every 3 days we have modified the regimen looking for the most effect dose and schedule. As a result of these experiments we have determined that 10mg/kg every 3 days has the ability to enhance vaccine therapy. In addition, it is becoming clear that we get the best results when we continue treatment beyond the initial 3 doses (see Figure 1 below). At this point we are delivering the drug I.P. However, (as mentioned as a possible alternative in our proposal), the data suggest that continuous infusion by ALZET pumps may enhance our effect even more. This remains as a possible future experiment, once we have derived data concerning the optimal delivery I.P.

Enhancing tumor vaccine therapy by preventing tumor-induced tolerance:

The basic vaccine protocol is shown in Figure 1 below. Under normal circumstances, tumor vaccines are ineffective in treating tumor in the neu-expressing mice. This is because the T cells from these mice are tolerant to the tumor antigens. The goal of our proposal is to inhibit tumor-induced tolerance pharmacologically with Go6976 and thus enhance the efficacy of the vaccine.

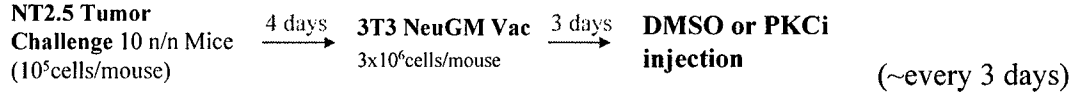

Figure 1

Previously, we demonstrated that by inhibiting PKC with Go6976 we could enhance immune function. Therefore a series of experiments were set up to determine if we could enhance vaccine efficacy in terms of survival by adding Go6976 to the vaccine protocol. Interestingly the tumor used for the challenge appeared to be more aggressive. That is, the mice were dying faster then previously reported (data not shown and Figure 3) (5). Of note, Dr. Elizabeth Jaffee's laboratory had been noting the same observations E. Jaffee personal communication). The enhanced aggressiveness of the tumor did not inhibit our experiments it just meant that the mice were that much more difficult to cure.

In addition, it is becoming increasingly clear that T regulatory cells have the ability to promote tumor-induced tolerance and inhibit vaccine function (6). This has also been found to be true in the *Neu* breast cancer model (Dr. Elizabeth Jaffee personal communication). It has long been known that cytoxan can enhance immune function and it is thought that this is due to the ability of this drug to eliminate T regulatory cells (7). Furthermore, vaccine in the setting of cytoxan have been shown to be marginally effective in this model. Thus, a series of experiments was performed to determine if Go6976 could enhance vaccine therapy + cytoxan.

Mice were treated as described in Figure 1 (10 per group) with either no vaccine, vaccine alone, vaccine+ cytoxan (10mg/kg IP on day 2), or vaccine+cytoxan+Go6976. The mice were evaluated for tumor size and survival.

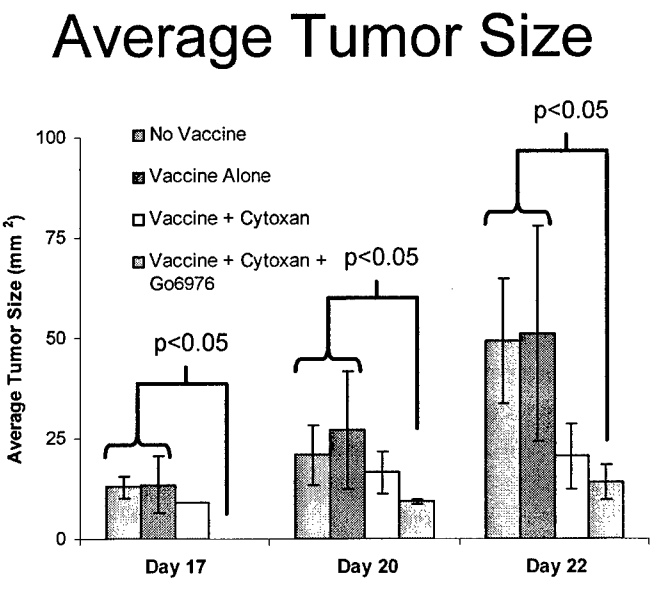

Figure 2: Average Tumor size: Neu-transgenic mice were challenged with tumor on day 0 and then treated as described in the legend above. Cytoxan was given on day 2 while vaccine was given on day 3. Go6976 was given every 3 days (days 6, 9 and 12)

As seen above at day 17 no tumor is evident in the Go6976 treated mice. Of note, there is a statistically significant difference in tumor growth between the vaccine+cytoxan+Go6976 group and the vaccine+cytoxan group. Thus, the effect is not due to the cytoxan but due to the addition of Go6976. The difference is still prevalent on Day 20 but loses significance on Day 22. At this time however, although tumor size in both the vaccine+cytoxan and the vaccine+cytoxan+Go6976 group are equivalent, they are still statistically smaller than the tumors in the vaccine alone group. Thus, as time goes on we lose the "Go6976 effect". Recall, the last dose of Go6976 was on day 12. Thus we interpret these findings as indicating that the addition of Go6976 can enhance vaccine efficacy (Day 17) early and that by continuing Go6976 treatment we might be able to prolong this effect.

In addition to tumor size we also examined the effect of Go6976 on survival. As seen below in Figure 3, the mice treated with vaccine+cytoxan+Go6976 had their survival curve shifted to the right when compared with the other treatments. Furthermore, the curve plateaued at 20% compared with 0% for the other groups. As mentioned above, normally the vaccine treated mice display around 15-30% survival so that for this experiment the conditions were more rigorous. Once again, the marked delay in death leads us to hypothesize that by continuing the treatment we might improve long term survival to a greater extent. We do not believe that the effect of Go6976 is due to the drug itself acting on the tumor because in previous experiments, drug alone did not have any beneficial effect (data not shown).

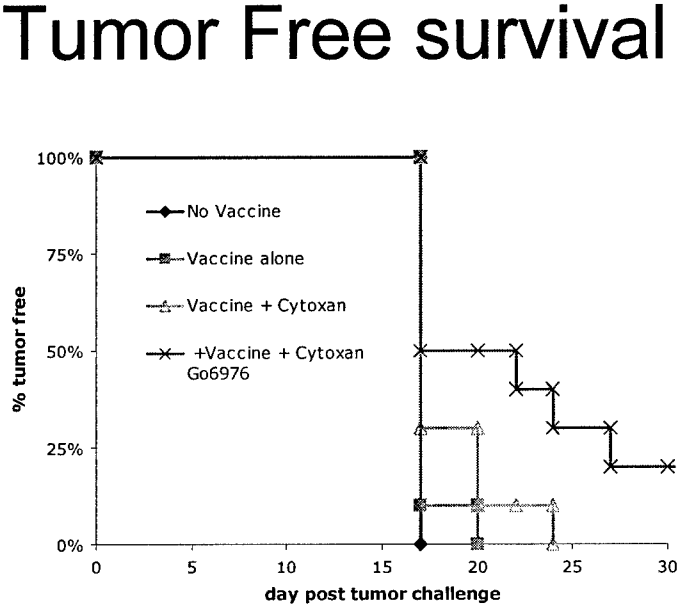

Figure 3: Tumor free survival: Mice were treated as described for Figure 2, as seen in the figure only the curve for the vaccine+cytoxan+Go6976 treated mice plateaued (20%).

Key research Accomplishments:

* Proof of the principle that the addition of Go6976 can enhance vaccine efficacy.
* Advances in terms of defining optimum dosing.
* Observation that a dose of cytoxan + G06976 can synergize in terms of enhancing vaccine therapy

Reportable outcomes:

1. Presentation of findings as a poster at "The Era of Hope" meeting

Conclusions:

Based on our observations that Go6976 could inhibit anergy induction *in vitro* we hypothesized that this PKC inhibitor could enhance the efficacy of tumor vaccine therapy in vivo. Our data clearly show that the addition of Go6976 to the vaccine protocol can delay tumor growth and enhance tumor free survival. These data serve to validate our hypothesis. Interestingly, in our series of experiments the tumor used was more aggressive (perhaps more realistic conditions) than previously published. Thus, these studies were performed under rigorous conditions. Indeed, while vaccine alone normally produces a 15-30% survival rate in our studies it was 0% (5).

An important conclusion from these studies is that we should continue Go6976 therapy for a longer period of time. Indeed, we hypothesize that the marked decrease in tumor size seen on day 17 would be maintained if we continued the drug. As such experiments are planned to test this hypothesis and in doing so further optimize our protocol.

The role of T regulatory cells in promoting tumor-induced tolerance is becoming greater appreciated (6). In this regard the combination of cytoxan and Go6976 appeared to be synergistic. Presumably, the cytoxan helped to eliminate T regulatory cells prior to the vaccine and then Go6976 helped to prevent the reinduction of tolerance after the vaccine. Along these lines, experiments are planned to demonstrate that the decreased tumor size and enhanced survival is due to an increase in tumor-specific T cells. In addition, we will also perform experiments to demonstrate that there is also a decrease in T regulatory cells.

These findings have important implications for the use of vaccines in preventing tumor development (Task 3). Indeed, not surprisingly, the optimal means of overcoming tumor-tolerance will be by inhibiting not only the induction of anergy but also inhibiting T regulatory cells.

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